THE ANALYSIS AND DETERMINATION OF PHENYL GLYCEROL ETHERS WITH PARTICULAR REFERENCE TO PHARMACEUTICAL PREPARATIONS

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Aromatic ethers of glycerol have for some time been known to possess interesting pharmacological properties[1,2]. The group of compounds is also interesting from a physicochemical standpoint in that the attachment of the glycerol molecule to the benzene nucleus produces a derivative soluble to some extent both in polar and in non-polar solvents. A general investigation of the group of compounds by Berger and Bradley[3,4,5] showed that αβ-dihydroxy-γ(2-methylphenoxy)-propane (myanesin, mephenesin) produced muscular relaxation and paralysis without causing respiratory arrest or influencing the blood pressure; the injection of the compound in anæsthesia was described by Mallinson[6], who advocated its use as a synthetic muscle-relaxing agent. Subsequently its use was reported in tetanus[7] and by mouth in the form of tablets and elixir for the treatment of spastic and hyperkinetic states by Berger and Schwartz[8].

Another compound in the same chemical group which has become available is α-*p*-chlorophenyl glyceryl ether (αβ-dihydroxy-γ-(*p*-chlorophenoxy)-propane, (gecophen, chlorphenesin), introduced by Hartley[9] as an antifungal agent. This is used in an ointment and in a dusting powder, while pessaries for the treatment of mycotic infection of the vagina have been used by Mackinlay[10]. It is clear that methods of analysis of such compounds, particularly with reference to their pharmaceutical preparations are required.

αβ-*Dihydroxy-γ-(2-methylphenoxy)-propane* (myanesin, mephenesin) forms colourless crystals m.pt. 70° to 71°C., soluble to the extent of approximately 1 per cent. in water at 22°C., very soluble in alcohol and propylene glycol; solutions have a neutral reaction and are stable to heat, light, acids and alkalis. A proposed D.A.K. specification[11] for the substance under the name glykresin has been published by the Control Laboratory of the Danish Pharmaceutical Society; this specification gives limit tests for acidity and alkalinity, for loss on drying, for ionised halogen, for sulphates, for heavy metals, for glycerol monochlorhydrin and in addition an assay depending on bromine absorption.

Published methods for the preparation of αβ-dihydroxy-γ(2-methylphenoxy) propane involve the condensation of glycerol and *o*-cresol in the presence of sodium acetate[12], or the action of glycerol monochlorhydrin on *o*-cresol[13]; if the compound is prepared by either of these routes *o*-cresol is a likely impurity and tests for its limitation should be included in any specification. The following test has proved to be satisfactory in the author's hands. To 0·1 g. dissolved in 5·5 ml. of water, add 3 ml. of a 4 per cent. solution of sodium hexametaphosphate, 1·5 ml. of Folin and Ciocalteau's reagent and 0·4 g. of anhydrous sodium carbonate. The mixture is heated in a water-bath for 5 minutes, cooled, and

the blue colour is compared with standards prepared from known amounts of *o*-cresol after allowance for the colour produced in a blank determination on the reagents used.

Among the assay processes available the following two have been used; the first, found to be the most useful, is based on a determination of the hydroxy-groups, while the second employs a periodic acid oxidation.

For hydroxy-groups.—Heat approximately 2 g. accurately weighed, with 20 ml. of a 15 per cent. solution of acetic anhydride in pyridine in a flask fitted with a reflux condenser and a ground glass joint on a water-bath for 2 hours; cool, add 40 ml. of water and titrate the free acid with N sodium hydroxide using phenolphthalein as indicator. A blank determination is performed at the same time omitting the substance under test; each ml. difference between the titrations is equivalent to 0·0911 g. of $C_{10}H_{12}O.(OH)_2$. Not less than 99·5 per cent. and not more than 100·5 per cent. should be indicated.

Assay using periodic acid. This is carried out according to the conditions given for the assay of propylene glycol in the National Formulary VIII; it has been found to be necessary to allow the period of contact with the periodic acid solution to be extended from the 15 minutes specified. In a few experiments a 24 hours' reaction time gave satisfactory results although this was not pursued in view of the reliability of the assay for hydroxyl groups.

p-*Chlorophenyl-α-glycerol ether, αβ-dihydroxy-γ*(p-*chlorophenoxy*) *propane* (gecophen, chlorphenesin) is a colourless crystalline solid, m.pt. 80°C. soluble in water to the extent of approximately 0·6 per cent. at 25°C., more readily soluble in organic solvents; aqueous solutions are neutral and are unaffected by dilute acids or alkalis or by exposure to light. The substance can be prepared[15] by the action of glycerol monochlorhydrin on *p*-chlorophenol and analytical determinations of purity should include limit tests for acidity and alkalinity, for loss on drying, for ionised halogen, for melting-point and for free phenols. The test for free phenols can be performed using Folin and Ciocalteau's procedure as outlined above for αβ-dihydroxy-γ-(2-methylphenoxy) propane. Three methods for the assay of *p*-chlorophenyl glycerol ether have proved to be satisfactory—an assay based on the determination of hydroxyl groups in the molecule, one based on the chlorine content, and a periodate oxidation process.

For hydroxyl groups. This can be carried out using the method described above for hydroxyl groups in mephenesin. Each ml. difference between the titrations is equivalent to 0·1013 g. of $C_9H_{11}O_3Cl$.

For halogen. Removal of the chlorine from the benzene nucleus can be effected quantitatively with sodium and amyl alcohol. Weigh accurately about 0·5 g. of sample into a flask of 250 ml. capacity, containing 50 ml. of amyl alcohol (AnalaR) and fitted with a wide bore air condenser. Pellets of sodium metal (about 2 g.) are added and the whole is warmed until a steady evolution of hydrogen takes place. When all the sodium has dissolved the liquid is refluxed gently for 2 hours, cooled, 80 ml. of water is added, followed by 40 ml. of nitric acid and 50 ml. of

0·1 N silver nitrate; the resulting mixture is cooled and back titrated with 0·1 N ammonium thiocyanate following the usual Volhard technique. Each ml. of 0·1 N of silver nitrate required is equivalent to 0·02026 g. of $C_9H_{11}O_3Cl$.

Assay by oxidation. This can be carried out as in the periodic acid assay of mephenesin, using the conditions given for the assay of propylene glycol[14]. Each ml. of sodium arsenite solution is equivalent to 0·01013 g. $C_9H_{11}O_3Cl$.

The Determination of Phenyl Glycerol Ethers in Pharmaceutical Preparations

The method of Titus, Ulick and Richardson[15] for the determination of αβ-dihydroxy-γ-(2-methylphenoxy) propane in body fluids and tissues involves coupling with diazotised 2:4-dinitroaniline, while an alternative procedure involves the colorimetric estimation of the formaldehyde

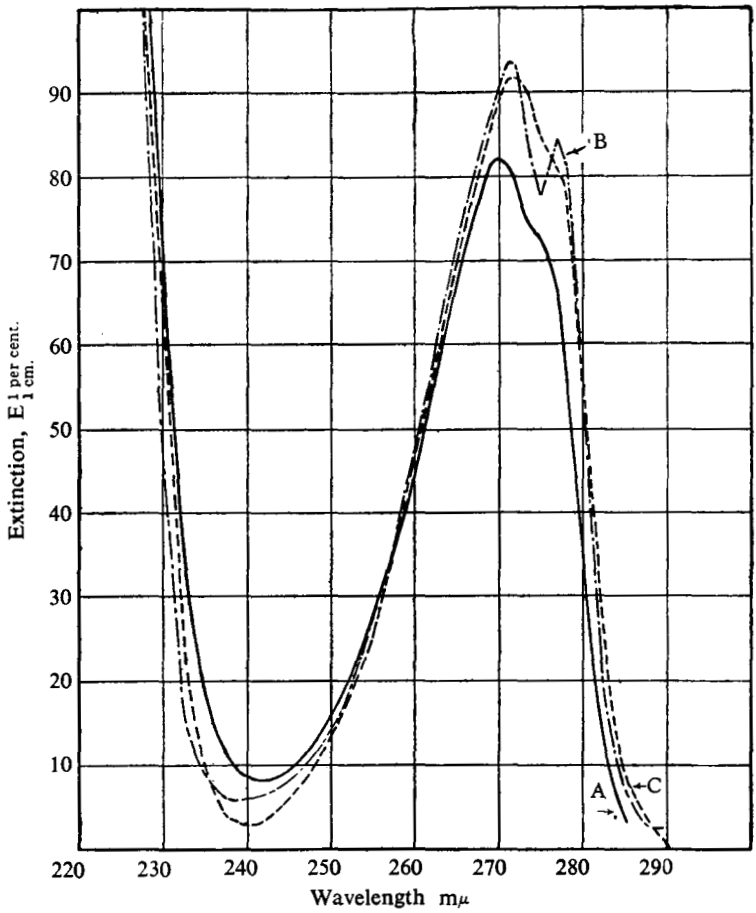

FIG. 1. Absorption spectra of mephenein. A, in water; B, in cyclohexane; C, in isopropyl alcohol.

resulting from periodate oxidation. Neither of these methods as given has been found to be reliable when applied to pharmaceutical preparations and considerable adaptation would be necessary to make them applicable.

The value of the determination of the ultra-violet absorption spectrum in the analysis of pharmaceutical preparations has become established in recent years. In preparations where there is an inert vehicle this method has much to recommend it as regards simplicity, accuracy, and speed of operation; with this in view an examination of the absorption spectrum of the two phenyl glycerol ethers was undertaken.

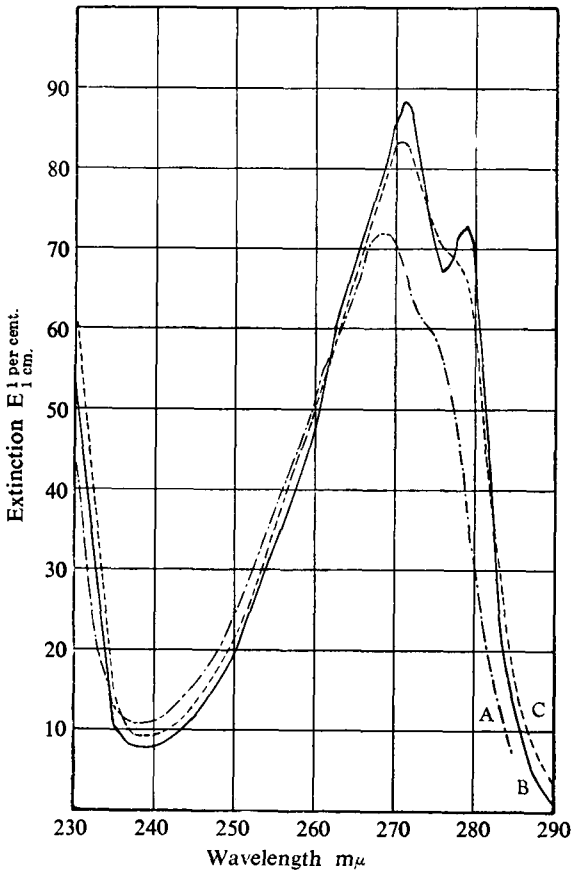

FIG. 2. Absorption spectra of chlorphenesin. A, in water; B, in cyclohexane; C, in isopropyl alcohol.

The spectrum of mephenesin in various solvents is shown in Fig. 1. A change of *p*H in aqueous medium did not produce a change in spectrum, an interesting point in view of the fact that the glycerol ethers generally are slightly more soluble in solutions of alkali hydroxides than in water; greater resolution of the peak is obtained in cyclohexane solution, two distinct maxima being visible.

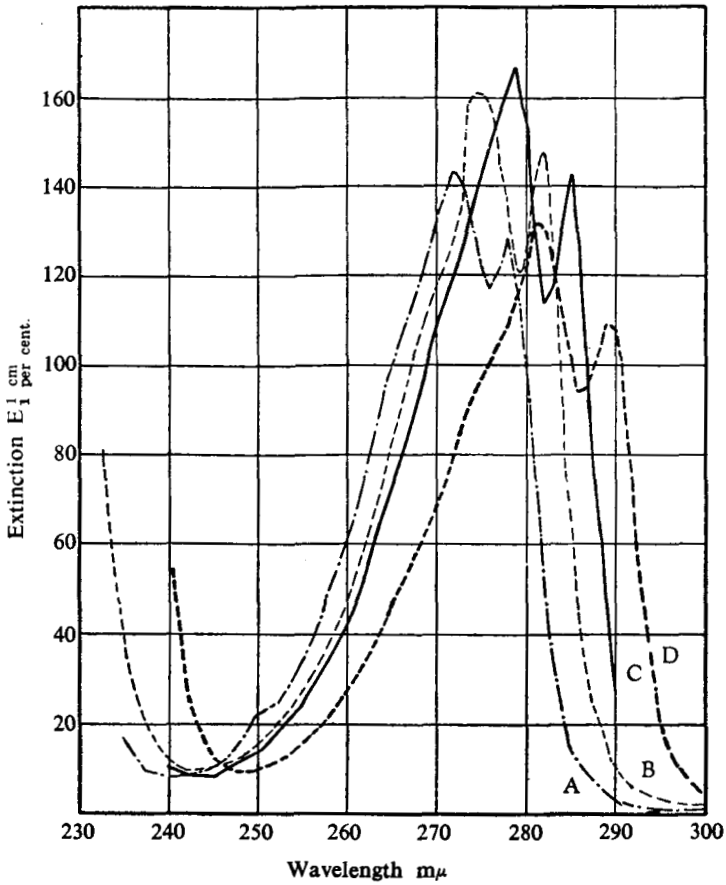

FIG. 3. Absorption spectra of phenyl ethers in cyclohexane. A, *o*-cresyl ethyl ether; B, *o*-chloroanisole; C, *p*-cresyl methyl ether; D, *p*-chloroanisole.

Chlorphenesin (Fig. 2) shows a similar absorption curve, although the combined effect of the halogen substituent coupled with a para-orientation has produced a shift of *ca.* 10 mμ in the peak wavelength. There is a complete analogy between chlorphenesin and mephenesin in the small relative peak differences between the various solvents; again cyclohexane produces greater resolution.

Generally the spectra of the above two compounds are those of substituted benzene derivatives where it has been shown that there is a constant ratio between the two peaks; in the spectra above, the shorter wavelength peak has not been realised. The actual maxima would, however, be below 220 mμ and would be of little value in pharmaceutical work owing to the strong end-absorption of most substances in this region. The glycerol moiety of the molecule plays little or no part in determining either the intensity of the absorption or the peak wavelength. To illustrate this point the spectra of a number of analogous benzene derivatives have been determined in which $-OCH_2-CHOH-CH_2OH$ has been

substituted by an alkyl group; these, in cyclohexane, are shown in Figure 3. It will be seen that *o*-cresyl-ethyl ether has a spectrum almost identical with that of mephenesin, the analogy being more apparent if the absorption maxima are calculated on a molecular basis. Para-substitution causes a shift to longer wavelength of *ca.* 7 mμ over the ortho-compound, a difference which is remarkably constant for both peaks.

TABLE I

	Solvent	λ max	$E^{1\%}_{1cm}$
Mephenesin	isopropyl alcohol	272·0 mμ	91·5
	cyclohexane	277·0 mμ	84·4
		271·5 mμ	93·5
	water	277·0 mμ	66·0
		270·0 mμ	82·1
Chlorphenesin	isopropyl alcohol	281·0 mμ	83·6
	cyclohexane	281·0 mμ	89·0
		289·0 mμ	73·2
p-Cresyl methyl ether	cyclohexane	285·0 mμ	142·2
		279·0 mμ	166·0
o-Cresyl ethyl ether	cyclohexane	278·0 mμ	128·0
		272·0 mμ	143·5
p-Chloranisole	cyclohexane	289·0 mμ	109·2
		281·5 mμ	131·3
o-Chloranisole	cyclohexane	282·0 mμ	148·0
		275·0 mμ	161·4

Elixirs of Mephenesin. A number of preparations of this compound have been formulated containing in the main propylene glycol or alcohol as solvents. In many cases where there are few absorbing impurities a direct determination of $E^{1\%}_{1cm}$ 270 mμ will yield a reliable result for the mephenesin content; in some formulations a correction for the solvent and flavouring may be necessary.

An alternative method of analysis is available in dilution with water, extraction with an immiscible solvent, and weighing the residue. Ether was found to give impure oily residues after extraction and was rejected; chloroform, however, gave surprisingly clean residues and although the pure compound is low in melting-point the extracted product was nearly always crystalline on cooling. The following method is suggested. A quantity of elixir (containing about 0·5 g. of mephenesin) is diluted to 50 ml. with water and extracted 5 times with 25 ml. quantities of chloroform. The resulting chloroform extracts after washing with 10 ml. water are bulked, evaporated to dryness, and dried at 100°C. to constant weight.

Injections of mephenesin are usually formulated with alcohol and/or propylene glycol; they can be treated similarly for analytical purposes.

Mephenesin Tablets. These can be extracted with chloroform, the chloroform washed to remove absorbing impurities and the absorption determined as before.

The estimation of *p*-chlorophenyl glycerol ether can be performed as indicated for mephenesin by the determination of $E^{1\%}_{1cm}$ 281 mμ;

this method is valid for simple aqueous solutions and for preparations which yield little irrelevant absorption on treatment. For more complex formulations, e.g., ointments and dusting powders, a different method must be used. Analysis will then depend on the constituents of the ointment or preparation; solvent extraction with a variety of solvents is usually most successful, followed by purification of the extract. The extracted chlorphenesin is then determined by weighing or by a determination of $E_{1\ cm.}^{1\ per\ cent.}$ on the final solution if this is sufficiently pure; the determination of total halogen content using sodium and amyl alcohol can also be used.

SUMMARY

1. The examination of αβ-dihydroxy-γ-(2-methylphenoxy) propane (myanesin, mephenesin) and of αβ-dihydroxy-γ-(*p*-chlorophenoxy) propane (gecophen, chlorphenesin) is described, a method based on the use of Folin and Ciocalteau's reagent being proposed for the detection of phenolic impurities.

2. Methods of assay based on acetylation or oxidation of the hydroxy groups are described and discussed.

3. The absorption spectra of phenyl glycerol ethers and related substances are described and their suitability for the determination of phenyl glycerol ethers in pharmaceutical preparations, is discussed.

4. Methods are proposed for the determination of mephenesin in injections, elixirs, and tablets and of chlorphenesin in ointments, dusting powders, and pessaries.

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